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Single-Molecule-Sensitive Fluorescent Sensors Based on Photoinduced Intramolecular Charge Transfer

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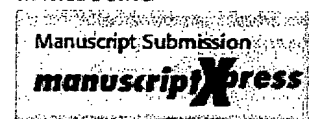
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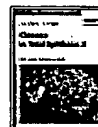
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TOP BOOKS
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Polarity (Potential) Sensitive Dye Based Mechanism

Applying this concept, the analyte concentration is measured by making use of so-called potential-sensitive dyes which actually respond to changes of the polarity of their environment [1]. This concept was first realized in the form of a PVC membrane containing plasticizer, valinomycin, and a potential-sensitive dye [2]. More recently, a fluorescent dye was positioned at the interface where the potential is created by placing a hydrophobic dye with a side chain as an anchor right at the PVC/water interface [3]. Various reasons have been given for the response of potential-sensitive dyes towards electrolytes [4]. Changes in membrane potential can occur as a result of charge separation across the membrane caused by the ionophore or by streaming potentials at the membrane surface caused by the sample solution passing by. Changes in surface potential result in optical changes which, in turn, may be due to a variety of effects: the Stark effect, where changes in the absorption and emission spectrum of a fluorophore take place when an external field is applied; changes in the otherwise homogeneous distribution of the fluorophore (aggregation at the interface); field-dependent distribution of the dye between regions of different polarity within the lipid membrane, resulting in a solvatochromic effect, such as hyperchromic or hypochromic shifts caused by the formation of di- or oligomeres. In the figure a schematic representation of the polymer/water interface of a potassium sensitive membrane based on a potential-sensitive dye [5] is given.
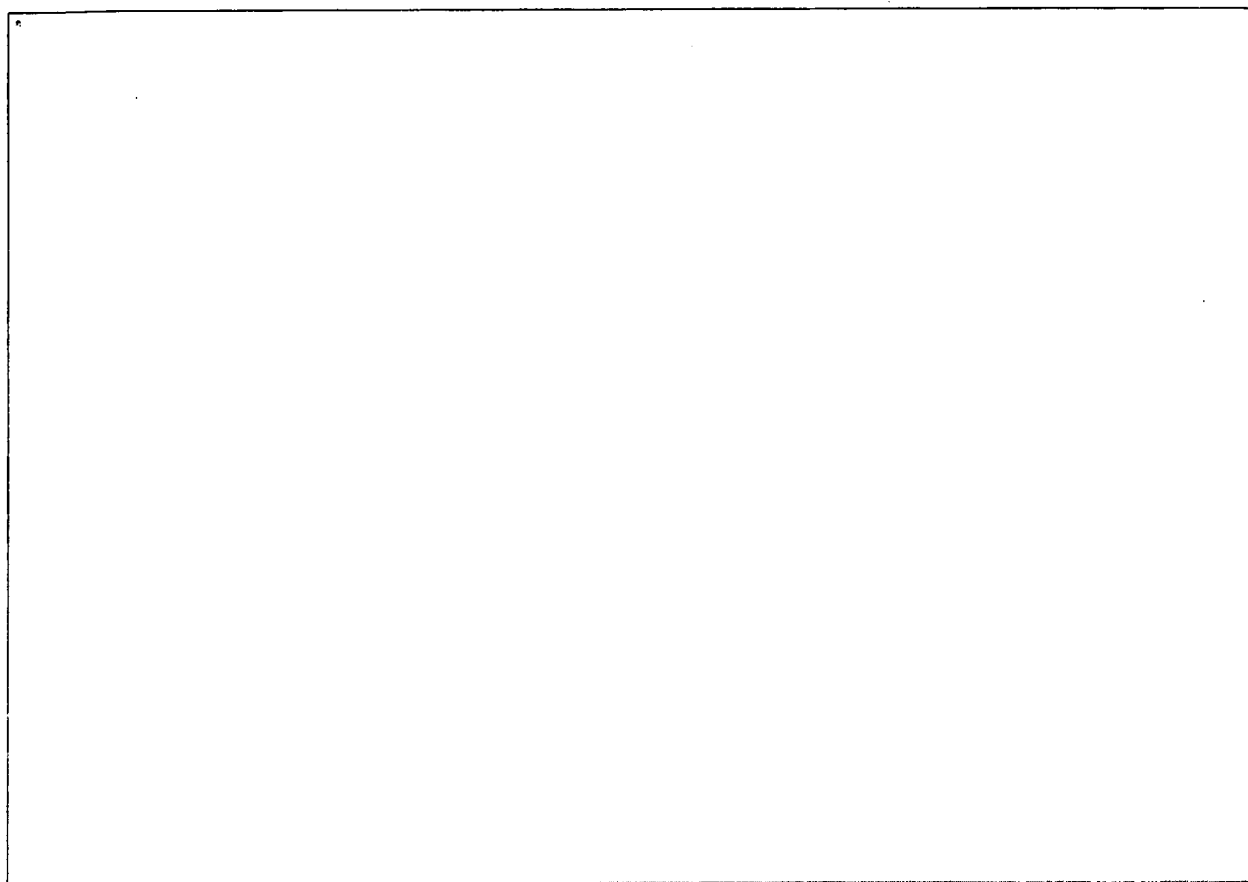
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Mechanism of acidification of the trans-Golgi network (TGN). In situ measurements of pH using retrieval of TGN38 and furin from the cell surface.

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Sorting of secretory cargo and retrieval of components of the biosynthetic pathway occur at the trans-Golgi network (TGN). The pH within the TGN is thought to be an important determinant of these functions. However, studies of the magnitude and regulation of the pH of the TGN have been hampered by the lack of appropriate detection methods. This report describes a noninvasive strategy to measure the luminal pH of the TGN in intact cells. We took advantage of endogenous cellular mechanisms for the specific retrieval of TGN resident proteins, such as TGN38 and furin, that transit briefly to the plasma membrane. Cells were transfected with chimeric constructs that contained the internalization and retrieval signals of TGN resident proteins, and a luminal (extracellular) epitope (CD25). Like TGN38 and furin, the chimeras were shown by fluorescence microscopy to accumulate within the TGN. During their transient exposure at the cell surface, the chimeras were labeled with extracellular anti-CD25 antibodies conjugated with a pH-sensitive fluorophore. Subsequent endocytosis and retrograde transport resulted in preferential labeling of the TGN with the pH-sensitive probe. Continuous, quantitative measurements of the pH of the TGN were obtained by ratio fluorescence imaging. The resting pH, calibrated using either ionophores or the "null point" technique, averaged 5.95 in Chinese hamster ovary cells and 5.91 in HeLa cells. The acidification was dissipated upon addition of concanamycin, a selective blocker of vacuolar-type ATPases. The counterion conductance was found to be much greater than the rate of H+ pumping at the steady state, suggesting that the acidification is not limited by an electrogenic potential. Both Cl- and K+ were found to contribute to the overall counterion permeability of the TGN. No evidence was found for the presence of active Na+/H+ or Ca2+/H+ exchangers on the TGN membrane. In conclusion, selective retrieval of recombinant proteins can be exploited to target ion-sensitive fluorescent probes to specific organelles. The technique provides real-time, noninvasive, and quantitative determinations of the pH, allowing the study of pH regulation within the TGN in intact cells. The acidic pH of the TGN reflects active H+ pumping into an organelle with a low intrinsic H+ permeability and a high conductance to monovalent ions.

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Section 13.5 — Other Nonpolar and Amphiphilic Probes

Amphiphilic Derivatives of Rhodamines, Fluoresceins and Coumarins

Each of our amphiphilic probes comprises a moderately polar fluorescent dye with a lipophilic "tail." When used to stain membranes, including liposomes, the lipophilic portion of the probe tends to insert in the membrane and the polar fluorophore resides on the surface (Figure 13.1), where it senses the membrane's surface environment and the surrounding medium.[1] Our lipophilic carbocyanines and styryl dyes (Section 13.4) are also amphiphilic molecules with a similar binding mode.

This section includes the classic membrane probes DPH, TMA-DPH, ANS, bis-ANS, TNS, prodan, laurdan and nile red, and also some lipophilic BODIPY and Dapoxyl dyes developed at Molecular Probes. Although they bear little resemblance to natural products, these probes tend to localize within cell membranes or liposomes or at their aqueous interfaces, where they are often used to report on characteristics of their local environment, such as viscosity, polarity and lipid order.

Octadecyl Rhodamine B

The relief of octadecyl rhodamine B (R18, O246; Figure 13.48) fluorescence self-quenching can be used to monitor membrane fusion [2–7] — one of several experimental approaches developed for this application that are described in lipid-mixing assays of membrane fusion (see Section 13.2). Octadecyl rhodamine B has been reported to undergo a potential-dependent "flip-flop" from one monolayer of a fluid-state phospholipid bilayer membrane to the other, with partial relief of its fluorescence quenching.[8,9] Investigators have used octadecyl rhodamine B in conjunction with video microscopy [10–12] or digital imaging techniques [13] to monitor viral fusion processes. Membrane fusion can also be followed by monitoring fluorescence resonance energy transfer [14] to octadecyl rhodamine B from an acylaminofluorescein donor such as 5-octadecanoylaminofluorescein [5,7,15,16] (O322). Fluorescence resonance energy transfer from fluorescein or dansyl labels to octadecyl rhodamine B has been used for structural studies of the blood coagulation factor IXa, EGF receptor and receptor-bound IgE.[17–19] Octadecyl rhodamine B has also been used to stain kinesin-generated membrane tubules,[20] to characterize detergent micelles,[21] to

assay for lysosomal degradation of lipoproteins [22] and to investigate the influence of proteins on lipid dynamics using time-resolved fluorescence anisotropy.[23]

Lipophilic Fluoresceins

The lipophilic fluorescein probes bind to membranes with the fluorophore at the aqueous interface and the alkyl tail protruding into the lipid interior (Figure 13.1). 5-Dodecanoylaminofluorescein (D109) is the hydrolysis product of our ImaGene Green C_{12}-FDG β-galactosidase substrate (D2893, Section 10.2). We also offer the homologous membrane probes, 5-hexadecanoyl- and 5-octadecanoylaminofluorescein [1,16,24] (H110, O322) and the octadecyl ester of fluorescein [25,26] (F3857).

Lipophilic fluorescein probes are commonly utilized for fluorescence recovery after photobleaching (FRAP) measurements of lipid lateral diffusion.[27] Some researchers have reported that 5-hexadecanoylaminofluorescein stays predominantly in the outer membrane leaflet of epithelia and does not pass through tight junctions, whereas the dodecanoyl derivative can "flip-flop" to the inner leaflet at 20°C (but not at <10°C) and may also pass through tight junctions.[28,29] More recent studies have indicated that the lack of tight junction penetration of 5-hexadecanoylaminofluorescein is due to probe aggregation rather than a significant difference in its transport properties.[30]

A Lipophilic Coumarin

4-Heptadecyl-7-hydroxycoumarin (H22730, Figure 13.49) is an alkyl derivative of the pH-sensitive blue-fluorescent 7-hydroxycoumarin (umbelliferone) fluorophore. As with other lipophilic coumarins,[31] 4-heptadecyl-7-hydroxycoumarin is primarily useful as a probe of membrane surfaces. Deprotonation of the 7-hydroxyl group is expected to be strongly dependent on membrane-surface electrostatic potential. The pK_a of 4-heptadecyl-7-hydroxycoumarin varies from 6.35 in the cationic detergent CTAB to 11.15 in the anionic detergent sodium dodecyl sulfate (SDS), as measured by its fluorescence response.[32] However, its pK_a in lipid assemblies is strongly dependent on the ionic composition of the membrane surface,[32,33] making it a sensitive probe of membrane-surface electrostatic potential.[34] 4-Heptadecyl-7-hydroxycoumarin has been used to measure pH differences at membrane interfaces in isolated plasma membranes of normal and multidrug-resistant murine leukemia cells.[35,36] 4-Heptadecyl-7-hydroxycoumarin has also been employed as a structural probe for the head-group region of phospholipid bilayers.[37]

DPH and DPH Derivatives

Diphenylhexatriene (DPH)

1,6-Diphenyl-1,3,5-hexatriene (DPH, D202; Figure 13.50) continues to be a popular fluorescent probe of membrane interiors. We also offer the cationic DPH derivatives TMA-DPH (T204, Figure 13.51) and TMAP-DPH (P3900), the anionic derivative DPH propionic acid (P459, Figure 13.52), as well as the phospholipid analog (D476, Section 13.2, Figure 13.19) and cholesteryl ester (C7794, Section 13.3). The orientation of DPH within lipid bilayers is loosely constrained. It is generally assumed to be oriented parallel to the lipid acyl chain axis (Figure 13.1), but it can also reside in the center of the lipid bilayer parallel to the surface, as demonstrated by time-resolved fluorescence anisotropy and polarized fluorescence measurements.[38-41] DPH shows no partition preference between coexisting gel- and fluid-phase phospholipids.[42] Intercalation of DPH and its derivatives into membranes is accompanied by strong enhancement of their fluorescence; their fluorescence is practically negligible in water. The fluorescence decay of DPH in lipid bilayers is complex.[43-45] Fluorescence decay data are often analyzed in terms of continuous lifetime distributions,[46-49] which are in turn interpreted as being indicative of lipid environment heterogeneity.

DPH and its derivatives are cylindrically shaped molecules with absorption and fluorescence emission transition dipoles aligned approximately parallel to their long molecular axis. Consequently, their fluorescence polarization is high in the absence of rotational motion and is very sensitive to reorientation of the long axis resulting from interactions with surrounding lipids. These properties have led to their extensive use for membrane fluidity measurements.[50] The exact physical interpretation of these measurements has some contentious aspects. For instance, the probes are largely sensitive to only the angular reorientation of lipid acyl chains — a motion that does not necessarily correlate with other dynamic processes such as lateral diffusion.[51] Reviews on this subject [40,50,52,53] should be consulted for further discussion. Time-resolved fluorescence polarization measurements of lipid order are more physically rigorous because they allow the angular range of acyl chain reorientation ("lipid order") to be resolved from its rate, and considerable research has been devoted to the interpretation of these measurements.[38,46,54,55]

TMA-DPH and TMAP-DPH

To improve the localization of DPH in the membrane, the derivative TMA-DPH (T204, Figure 13.51) was introduced, which contains a cationic trimethylammonium substituent that acts as a surface anchor.[56-58] A newer version of this probe, TMAP-DPH (P3900, Figure 16.23), incorporates an additional three-carbon spacer between the fluorophore and the trimethylammonium substituent.[56] Like DPH, these derivatives readily partition from aqueous dispersions into membranes and other lipid assemblies, accompanied by strong

fluorescence enhancement. The lipid–water partition coefficients (K_p) for TMA-DPH and TMAP-DPH (K_p = 2.4 × 10^5 and 2.9 × 10^5, respectively) are lower than for DPH (K_p = 1.3 × 10^6), reflecting the increased water solubility caused by their polar substituents.[59] The partitioning properties of TMAP-DPH in multilamellar liposomes have been described.[56,60,61] The fluorescence decay lifetime of TMA-DPH is more sensitive to changes in lipid composition and temperature than is the fluorescence decay lifetime of DPH.[62–64]

Staining of cell membranes by TMA-DPH is much more rapid than staining by DPH. However, the duration of plasma membrane surface staining by TMA-DPH before internalization into the cytoplasm is quite prolonged.[65,66] As a consequence, TMA-DPH introduced into Madin–Darby canine kidney (MDCK) cell plasma membranes does not diffuse through tight junctions and remains in the apical domain, whereas the anionic DPH propionic acid (P459, Figure 13.52) accumulates rapidly in intracellular membranes.[67] TMA-DPH residing in the plasma membrane can be extracted by washing with medium, thus providing a method for isolating internalized probe and monitoring endocytosis [68] (Section 16.1). Furthermore, because TMA-DPH is virtually nonfluorescent in water and binds in proportion to the available membrane surface,[69] its fluorescence intensity is sensitive to increases in plasma membrane surface area resulting from exocytosis.[68,70,71]

TMA-DPH fluorescence polarization measurements can be combined with video microscopy to provide spatially resolved images of phospholipid order in large liposomes and single cells.[72–75] Information regarding lipid order heterogeneity among cell populations can be obtained in a similar way using flow cytometry.[76–78]

DPH Propionic Acid

DPH propionic acid (P459, Figure 13.52) has principally been used as a synthetic intermediate for preparation of phospholipids (for example, D476; Section 13.2) and other molecules. When used as a membrane probe, its location within the lipid bilayer interior is dependent on the ionization state of its carboxylate group.[56] DPH propionic acid has been used together with DPH as a probe for lipid–protein interactions [79] and ethanol-induced perturbations of membrane structure.[80]

Nonpolar BODIPY Probes

Molecular Probes' nonpolar derivatives of the BODIPY fluorophore offer an unusual combination of nonpolar structure (Figure 13.53) and long-wavelength absorption and fluorescence.[81] These dyes have potential applications as stains for neutral lipids and as tracers for oils and other nonpolar liquids. Staining with the BODIPY 493/503 dye (D3922) has been shown by flow cytometry to be more specific for

cellular lipid droplets than staining with nile red [82] (N1142). The low molecular weight of the BODIPY 493/503 dye (262 daltons) results in the probe having a relatively fast diffusional rate in membranes.[83] BODIPY 505/515 (D3921) rapidly permeates cell membranes of live zebrafish embryos,[84,85] selectively staining cytoplasmic yolk platelets. This staining provides dramatic contrast enhancement of cytoplasm relative to nucleoplasm and interstitial spaces, allowing individual cell boundaries and cell nuclei to be imaged clearly with a confocal laser-scanning microscope (Figure 13.2, Figure 13.54). The very long–wavelength BODIPY 665/676 dye (B3932) has fluorescence that is not visible to the human eye; however, it has found use as a probe for reactive oxygen species [86] (Section 19.2). BODIPY FL C_5-ceramide (D3521, B22650; Section 13.3) stains the plasma membrane, Golgi apparatus and cytoplasmic particles within the superficial enveloping layer (EVL) of the embryos. However, once the fluorescent lipid percolates through the EVL epithelium, it remains localized within the interstitial fluid of the embryo and freely diffuses between cells (Figure 13.55). Vital staining with BODIPY FL C_5-ceramide thus allows hundreds of cells to be imaged *en masse* during morphogenetic movements.[84,87] BODIPY dyes have small fluorescence Stokes shifts, extinction coefficients that are typically greater than 80,000 $cm^{-1}M^{-1}$ and high fluorescence quantum yields that are not diminished in water.[88] Their photostability is generally high; this, together with other favorable characteristics (very low triplet–triplet absorption), results in excellent performance of the BODIPY 493/503 and BODIPY 505/515 dyes as flashlamp-pumped laser dyes.[89,90]

Pyrene, Nile Red and Bimane Probes

Nonpolar Pyrene and NBD Probes

1,3-Bis-(1-pyrene)propane (B311) has two pyrene moieties linked by a three-carbon alkylene spacer. This probe is somewhat analogous to the bis-pyrenyl phospholipids (Section 13.2) in that excimer formation (and, consequently, the fluorescence emission wavelength) is controlled by intramolecular rather than bimolecular interactions. Thus, the probe is highly sensitive to constraints imposed by its environment, and can therefore be used as a viscosity sensor for interior regions of lipoproteins, membranes, micelles, liquid crystals and synthetic polymers.[91,92] Because excimer formation results in a spectral shift (Figure 13.8), the probe may be useful for ratio imaging of molecular mobility.[93] However, pyrene fatty acids (Section 13.2) appear to be preferable for this purpose because the uptake of 1,3-bis-(1-pyrene)propane by cells is limited.

Nile Red

The phenoxazine dye nile red (N1142, Figure 13.56) is used to localize and quantitate lipids, particularly neutral lipid droplets within cells.[82,94–96] It is

selective for neutral lipids such as cholesteryl esters [97,98] (and also, therefore, for lipoproteins) and is suitable for staining lysosomal phospholipid inclusions.[99] Nile red is almost nonfluorescent in water and other polar solvents but undergoes fluorescence enhancement and large absorption and emission blue shifts in nonpolar environments.[100,101] Its fluorescence enhancement upon binding to proteins is weaker than that produced by its association with lipids [101] (Figure 13.57). Ligand-binding studies on tubulin and tryptophan synthase [102] have exploited the environmental sensitivity of nile red's fluorescence. Nile red has also been used to detect sphingolipids on thin-layer chromatograms [103] and to stain proteins after SDS-polyacrylamide gel electrophoresis.[104]

Bimane Azide

Bimane azide (B30600, Section 5.3) is a small blue-fluorescent reagent (excitation/emission maxima ~375/458 nm) for photoaffinity labeling of proteins, potentially including membrane proteins. This reactive fluorophore's small size may reduce the likelihood that the label will interfere with the function of the biomolecule, an important advantage for site-selective probes.

Membrane Probes with Environment-Sensitive Spectral Shifts

Prodan and Laurdan

Prodan (P248, Figure 13.58), introduced by Weber and Farris in 1979, has both electron-donor and electron-acceptor substituent, resulting in a large excited-state dipole moment and extensive solvent polarity–dependent fluorescence shifts [105] (Figure 13.59). Several variants of the original probe have since been prepared, including the lipophilic derivative laurdan (D250, Figure 13.60) and thiol-reactive derivatives acrylodan and badan (A433, B6057; Section 2.3), which can be used to confer the environment-sensitive properties of this fluorophore on bioconjugates. When prodan or its derivatives are incorporated into membranes, their fluorescence spectra are sensitive to the physical state of the surrounding phospholipids.[106] In membranes, prodan appears to localize at the surface,[107] although Fourier transform infrared (FTIR) measurements indicate some degree of penetration into the lipid interior.[108] Excited-state relaxation of prodan is sensitive to the nature of the linkage (ester or ether) between phospholipid hydrocarbon tails and the glycerol backbone.[109] In contrast, laurdan's excited-state relaxation is independent of head-group type, and is instead determined by water penetration into the lipid bilayer.[110,111] Two-photon infrared excitation techniques have been successfully applied to both prodan and laurdan, although both probes nominally require ultraviolet excitation (~360 nm).[112–115]

Much experimental work using these probes has sought to characterize coexisting

lipid domains based on their distinctive fluorescence spectra,[113,116–120] an approach that is intrinsically amenable to dual-wavelength ratio measurements.[111,121] Other applications include detecting nonbilayer lipid phases,[122,123] mapping changes in membrane structure induced by cholesterol and alcohols [124–127] and assessing the polarity of lipid/water interfaces.[128,129] Like ANS (see below), prodan is also useful as a noncovalently interacting probe for proteins.[130–133]

Dapoxyl Derivatives

Molecular Probes has developed a variety of probes based on our Dapoxyl fluorophore.[134] Dapoxyl sulfonamide derivatives exhibit UV absorption with maxima near 370 nm and extinction coefficients >24,000 $cm^{-1}M^{-1}$. In polar solvents, Dapoxyl sulfonamides have Stokes shifts in excess of 200 nm (Figure 1.108). In nonpolar environments, the emission may become structured and shift to much shorter wavelengths (Figure 13.61). Dapoxyl butylsulfonamide (D12801, Figure 13.62) is a neutral probe that is expected to partition into membranes, whereas Dapoxyl sulfonic acid [135] (D12800) is expected to localize at the aqueous interface of membranes. Reactive versions of the Dapoxyl probes are described in Section 1.7, Section 2.3 and Section 3.3.

Anilinonaphthalenesulfonate (ANS) and Related Derivatives

The use of anilinonaphthalene sulfonates (ANS) as fluorescent probes dates back to the pioneering work of Weber in the 1950s, and this class of probes remains valuable for studying both membrane surfaces and proteins. Slavik's 1982 review of its properties is recommended reading, especially for the extensive compilation of spectral data.[136] The primary member of this class, 1,8-ANS (A47, Figure 13.63), and its analogs 2,6-ANS (A50) and 2,6-TNS (T53) are all essentially nonfluorescent in water, only becoming appreciably fluorescent when bound to membranes (quantum yields ~0.25) or proteins (quantum yields ~0.7) [136–138] (Figure 13.57). This property makes them sensitive indicators of protein folding (see "Monitoring Protein-Folding Processes with Anilinonaphthalenesulfonate Dyes"), conformational changes [139–142] and other processes that modify the exposure of the probe to water. Fluorescence of 2,6-ANS is also enhanced by cyclodextrins, permitting a sensitive method for separating and analyzing cyclodextrins with capillary electrophoresis.[143]

Bis-ANS

Bis-ANS (B153, Figure 13.64) is superior to 1,8-ANS as a probe for nonpolar cavities in proteins, often binding with an affinity that is orders-of-magnitude higher.[144–147] Bis-ANS has particularly high affinity for nucleotide binding sites of

some proteins.[148–150] It is also useful as a structural probe for tubulin [151,152] and as an inhibitor of microtubule assembly.[153–155] Covalent photoincorporation of bis-ANS into proteins has been reported.[156]

DCVJ

The styrene derivative DCVJ (D3923) is a sensitive indicator of tubulin assembly and actin polymerization.[157,158] The fluorescence quantum yield of DCVJ is strongly dependent on environmental rigidity, resulting in large fluorescence increases when the dye binds to antibodies [159] and when it is compressed in synthetic polymers or phospholipid membrane interiors.[160,161] DCVJ has been used for microviscosity measurements of phospholipid bilayers.[161]

4-Amino-4'-Benzamidostilbene-2,2'-Disulfonic Acid

The disodium salt of 4-amino-4'-benzamidostilbene-2,2'-disulfonic acid (MBDS or BADS, A11760) has much lower residual fluorescence in water than does ANS. Upon binding to the hydrophobic pockets of certain proteins, its fluorescence is strongly enhanced.[162,163] The nearly 100-fold increase in MBDS's fluorescence upon forming a 1:1 complex with human serum albumin is useful for quantitation of that protein.[162] The compound is also an inhibitor of erythrocyte band 3 and other anion transporters [163,164] (Section 16.3).

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(1989); 46. Biophys Chem 48, 337 (1994); 47. Biochim Biophys Acta 1104, 273 (1992); 48. Biochemistry 29, 3248 (1990); 49. Chem Phys Lipids 50, 1 (1989); 50. Chem Phys Lipids 64, 99 (1993); 51. Biochim Biophys Acta 649, 471 (1981); 52. Chem Phys Lipids 64, 117 (1993); 53. Biochim Biophys Acta 854, 38 (1986); 54. Chem Phys 185, 393 (1994); 55. Chem Phys Lett 216, 559 (1993); 56. Biochemistry 37, 8180 (1998); 57. Biochemistry 27, 7723 (1988); 58. Biochemistry 20, 7333 (1981); 59. Biochem Biophys Res Commun 181, 166 (1991); 60. Chem Phys Lipids 66, 135 (1993); 61. J Fluorescence 3, 145 (1993); 62. Chem Phys Lipids 55, 29 (1990); 63. Biochemistry 26, 5121 (1987); 64. Biochemistry 26, 5113 (1987); 65. Biochim Biophys Acta 845, 60 (1985); 66. Cell Biophys 5, 129 (1983); 67. Am J Physiol 255, F22 (1988); 68. Cell Biology: A Laboratory Handbook, 2nd Ed., Vol. 2, Celis JE, Ed. pp. 63-69 (1998); 69. Biochemistry 25, 2149 (1986); 70. J Cell Biol 135, 1741 (1996); 71. Biochim Biophys Acta 1147, 194 (1993); 72. Fluorescent and Luminescent Probes for Biological Activity, Mason WT, Ed. pp. 420-425 (1993); 73. Am J Physiol 260, C1 (1991); 74. FASEB J 5, 2078 (1991); 75. Biophys J 57, 1199 (1990); 76. Cytometry 39, 151 (2000); 77. Biochim Biophys Acta 1067, 71 (1991); 78. Plant Physiol 94, 729 (1990); 79. Biochemistry 37, 16653 (1998); 80. Biochemistry 34, 5945 (1995); 81. J Photochem Photobiol A 121, 177 (1999); 82. Cytometry 17, 151 (1994); 83. Biophys J 71, 2656 (1996); 84. Methods Mol Biol 122, 185 (1999); 85. Neuron 20, 1081 (1998); 86. J Agric Food Chem 48, 1150 (2000); 87. Methods Cell Biol 59, 179 (1999); 88. Anal Biochem 198, 228 (1991); 89. Heteroatomic Chem 1, 389 (1990); 90. Optics Comm 70, 425 (1989); 91. Arch Pharm Res 20, 1 (1997); 92. Biochim Biophys Acta 1149, 86 (1993); 93. Eur J Cell Biol 65, 172 (1994); 94. J Histochem Cytochem 45, 743 (1997); 95. J Cell Biol 123, 1567 (1993); 96. Exp Cell Res 199, 29 (1992); 97. J Cell Biol 108, 2201 (1989); 98. J Chromatogr 421, 136 (1987); 99. Histochemistry 97, 349 (1992); 100. Anal Chem 62, 615 (1990); 101. Anal Biochem 167, 228 (1987); 102. J Biol Chem 270, 6357 (1995); 103. Anal Biochem 208, 121 (1993); 104. Biotechniques 21, 625 (1996); 105. Photochem Photobiol 58, 499 (1993); 106. Photochem Photobiol 70, 557 (1999); 107. Biochemistry 27, 399 (1988); 108. Biochemistry 28, 8358 (1989); 109. Biochemistry 29, 11134 (1990); 110. Biophys J 66, 763 (1994); 111. Biophys J 60, 179 (1991); 112. Anal Chem 73, 2302 (2001); 113. Biophys J 78, 290 (2000); 114. Biophys J 77, 2090 (1999); 115. Biophys J 72, 2413 (1997); 116. Biophys J 80, 1417 (2001); 117. Biophys J 66, 120 (1994); 118. Photochem Photobiol 57, 420 (1993); 119. Biophys J 57, 1179 (1990); 120. J Biol Chem 265, 20044 (1990); 121. Photochem Photobiol 57, 403 (1993); 122. Biochemistry 31, 1550 (1992); 123. Biophys J 57, 925 (1990); 124. Biochim Biophys Acta 1511, 330 (2001); 125. Biophys J 68, 1895 (1995); 126. Biophys J 65, 1404 (1993); 127. Biochemistry 31, 9473 (1992); 128. J Biol Chem 269, 10298 (1994); 129. J Biol Chem 269, 7429 (1994); 130. Biochemistry 37, 7167 (1998); 131. Biochem J 290, 411 (1993); 132. Eur J Biochem 204, 127 (1992); 133. Nature 319, 70 (1986); 134. Photochem Photobiol 66, 424 (1997); 135. J Photochem Photobiol A 131, 95 (2000); 136. Biochim Biophys Acta 694, 1 (1982); 137. Biophys J 74, 422 (1998); 138. Biochemistry 7, 3381 (1968); 139. Biochemistry 38, 2110 (1999); 140.

Biochemistry 37, 4621 (1998); 141. Biochemistry 37, 13862 (1998); 142. Biochemistry 37, 16802 (1998); 143. J Chromatogr A 680, 233 (1994); 144. Arch Biochem Biophys 268, 239 (1989); 145. Biochemistry 24, 3852 (1985); 146. Biochemistry 24, 2034 (1985); 147. Biochemistry 8, 3915 (1969); 148. Biochemistry 31, 2982 (1992); 149. Biochim Biophys Acta 1040, 66 (1990); 150. Proc Natl Acad Sci U S A 74, 2334 (1977); 151. Biochemistry 37, 4687 (1998); 152. Biochemistry 33, 11891 (1994); 153. Biochemistry 37, 17571 (1998); 154. Biochemistry 31, 6470 (1992); 155. J Biol Chem 259, 14647 (1984); 156. Biochemistry 34, 7443 (1995); 157. Anal Biochem 204, 110 (1992); 158. Biochemistry 28, 6678 (1989); 159. Biochemistry 32, 7589 (1993); 160. Chem Phys 169, 351 (1993); 161. Biochemistry 25, 6114 (1986); 162. Biochim Biophys Acta 229, 547 (1971); 163. Am J Physiol 259, C439 (1990); 164. Biochemistry 31, 12610 (1992).

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Monitoring Protein-Folding Processes with Anilinonaphthalenesulfonate Dyes

1,8-ANS (A47) and bis-ANS (B153) have proved to be sensitive probes for partially folded intermediates in protein-folding pathways (Table). The basis of these applications is the strong fluorescence enhancement exhibited by these amphiphilic dyes when their exposure to water is lowered (Figures 1 and 2). Consequently, fluorescence of ANS increases substantially when proteins to which it is bound undergo transitions from unfolded to fully or partially folded states that provide shielding from water. Molten globule intermediates are characterized by particularly high ANS fluorescence intensities due to the exposure of hydrophobic core regions that are inaccessible to the dye in the native structure.[1] Binding of 1,8-ANS and bis-ANS to proteins is noncovalent and involves a combination of electrostatic and hydrophobic modes.[2-6] Some investigators have noted that the dye-binding event itself may induce protein conformational changes, indicating the advisability of correlating ANS fluorescence measurements with data obtained using other physical techniques.[5,7-9] In particular, high-resolution structural analysis of an ANS–protein complex by X-ray crystallography has demonstrated the occurrence of local rearrangements of the protein structure to accommodate the dye.[10]

Table. Applications of ANS and bis-ANS as indicators of protein folding.

Protein	Reference
Actin	Biochemistry 38, 6261 (1999)
Carbonic anhydrase	Biochemistry 40, 2653 (2001)
Cathepsin	Biochemistry 39, 12382 (2000)

α- and β-crystallins	Biochemistry 39, 1420 (2000); J Biol Chem 275, 4565 (2000); J Biol Chem 275, 22009 (2000)
Cytochrome c	Biochemistry 38, 13635 (1999)
Glucose/xylose isomerase	Eur J Biochem 267, 6331 (2000)
Glutathione transferase	Biochemistry 39, 12336 (2000); Biochemistry 38, 16686 (1999)
GroEL	Biochemistry 40, 4484 (2001); J Biol Chem 275, 33504 (2000); J Mol Biol 276, 505 (1998)
Human serum albumin (HSA)	Biochim Biophys Acta 1476, 139 (2000); Eur J Biochem 266, 26 (1999)
α-lactalbumin	Biochemistry 40, 2138 (2001); Proteins 42, 237 (2001)
β-lactoglobulin	Biochemistry 39, 3565 (2000); Eur J Biochem 267, 3957 (2000)
Lysozyme	Biochemistry 39, 3248 (2000); Biochemistry 37, 6772 (1998)
β2-microglobulin	J Mol Biol 307, 379 (2001); Biochemistry 39, 8735 (2000)
Phospholipase A2	Biochemistry 38, 2919 (1999)
Ricin	J Biol Chem 275, 9263 (2000)
α-synuclein	J Biol Chem 276, 10737 (2001)
Rhodanese	J Biol Chem 275, 33504 (2000); J Biol Chem 274, 33795 (1999)

Figure 1. Fluorescence emission spectra of equal concentrations of 1,8-ANS (A47) in ethanol:water mixtures. The labels adjacent to each curve indicate the percentage of ethanol in the solvent mixture.
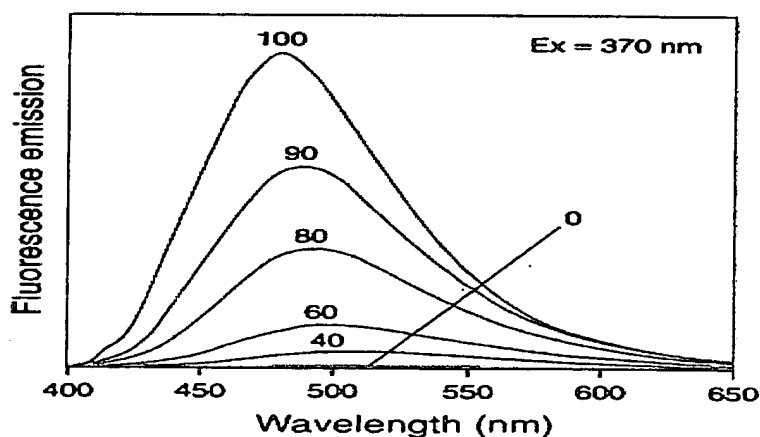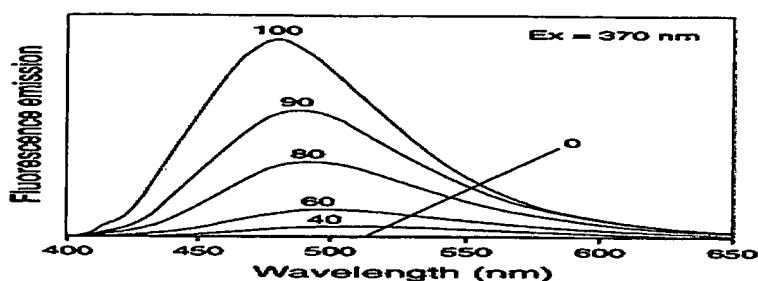
Figure 2. Fluorescence enhancement of 1,8-ANS (1-anilinonaphthalene-8-sulfonic acid, A47) upon binding to protein. The image shows aqueous solutions of 1,8-ANS excited by ultraviolet light. Addition of protein (bovine serum albumin) to the solution in the cuvette on the left results in intense blue fluorescence. In comparison, the fluorescence of uncomplexed free dye in the cuvette on the right is negligible.
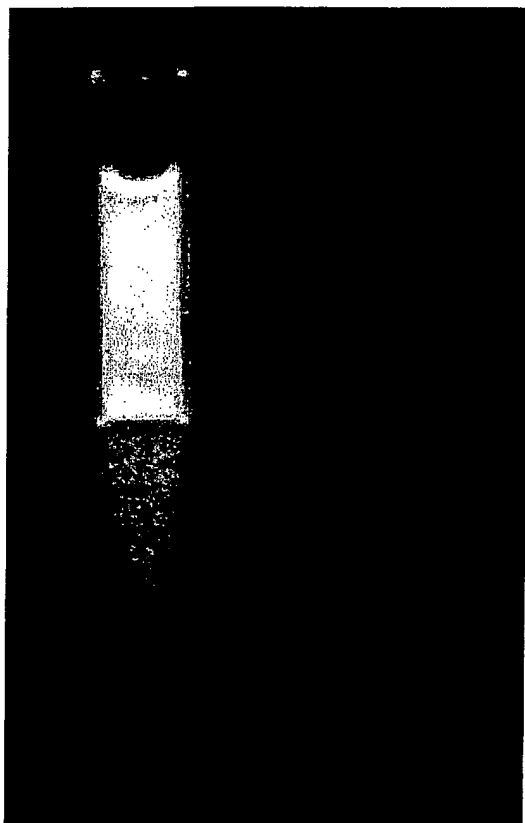
1. Biopolymers 31, 119 (1991); **2.** Biophys J 74, 422 (1998); **3.** Biophys J 70, 69 (1996); **4.** Biochemistry 33, 7536 (1994); **5.** Biopolymers 49, 451 (1999); **6.** Biochem J 282, 589 (1992); **7.** Biochemistry 38, 13635 (1999); **8.** Biophys J 75, 2195 (1998); **9.** Biochemistry 40, 4484 (2001); **10.** Proc Natl Acad Sci U S A 97, 6345 (2000).

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Figure 13.48 O246 octadecyl rhodamine B chloride.

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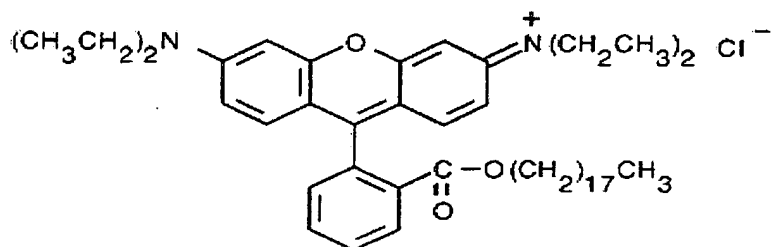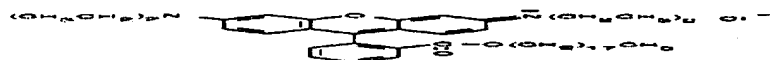
Figure 13.49 H22730 4-heptadecyl-7-hydroxycoumarin.

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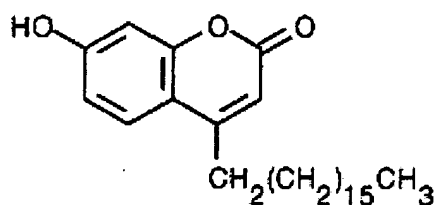
Figure 13.50 D202 1,6-diphenyl-1,3,5-hexatriene.

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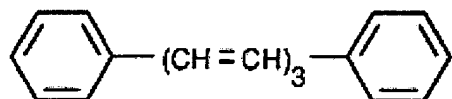
Figure 13.51 T204 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene
p-toluenesulfonate.

* * * * *
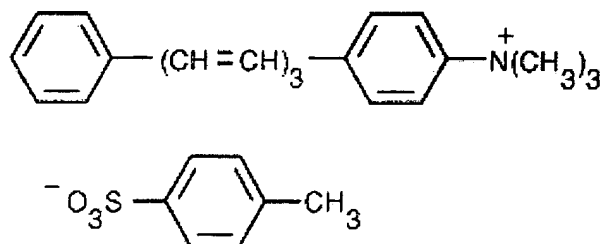
Figure 13.52 P459 3-(4-(6-phenyl)-1,3,5-hexatrienyl)phenylpropionic acid.

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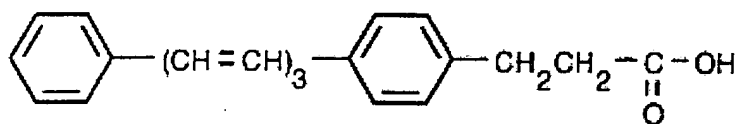
Figure 13.53 D3922
4,4-difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-*s*-indacene.

* * * * *
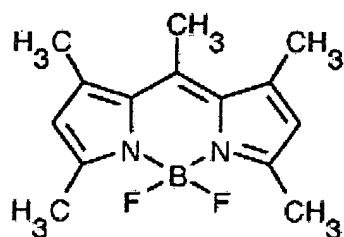
Figure 13.54 Dorsal view of the midbrain/hindbrain region of a 15-somite stage zebrafish embryo labeled with BODIPY 505/515 (D3921). BODIPY 505/515 localizes in lipidic yolk platelets, producing selective cytoplasmic staining. This pseudocolored confocal image was obtained using a Bio-Rad MRC-600 microscope. Image contributed by Mark Cooper, University of Washington.

* * * * *
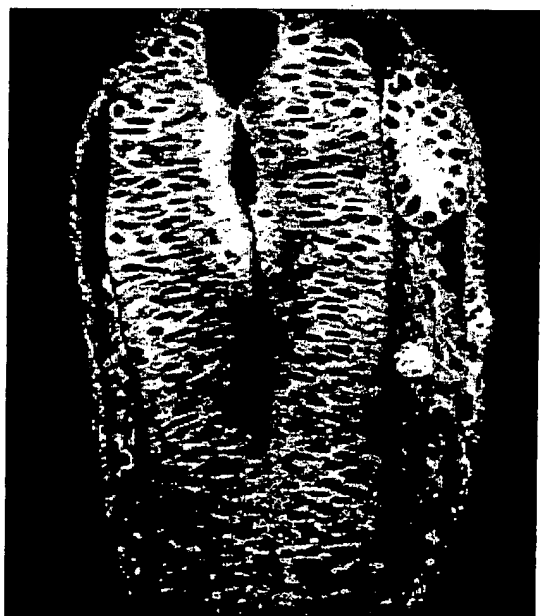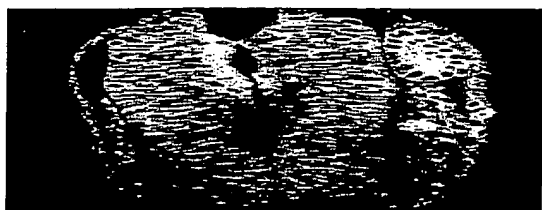
Figure 13.55 Cells in the notochord rudiment of a zebrafish embryo undergoing mediolateral intercalation to lengthen the forming notochord. BODIPY FL C5-ceramide (D3521) localizes in the interstitial fluid of the zebrafish embryo and freely diffuses between cells, illuminating cell boundaries. This confocal image was obtained using a Bio-Rad MRC-600 microscope. Image contributed by Mark Cooper, University of Washington.

* * * * *
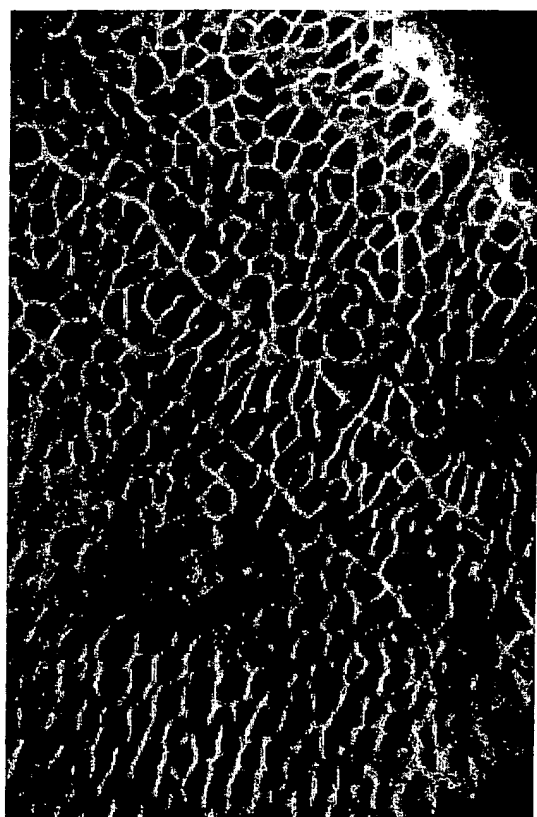
Figure 13.56 N1142 nile red.

(CH₃CH₂)₂N —

* * * * *
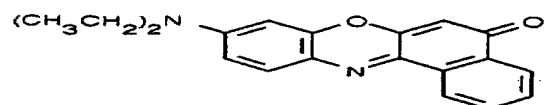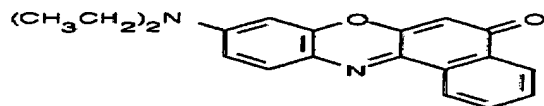
Figure 13.57 Fluorescence emission spectra of A) 1,8-ANS (A47) and B) nile red (N1142) bound to protein and phospholipid vesicles. Samples comprised 1 µM dye added to 20 µM bovine serum albumin (BSA) or 100 µM dioctadecenoylglycerophosphocholine (DOPC).

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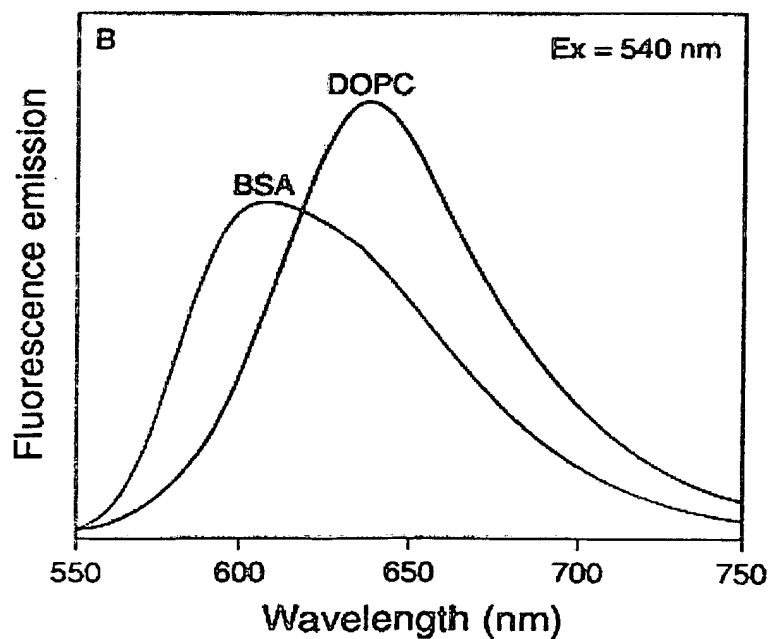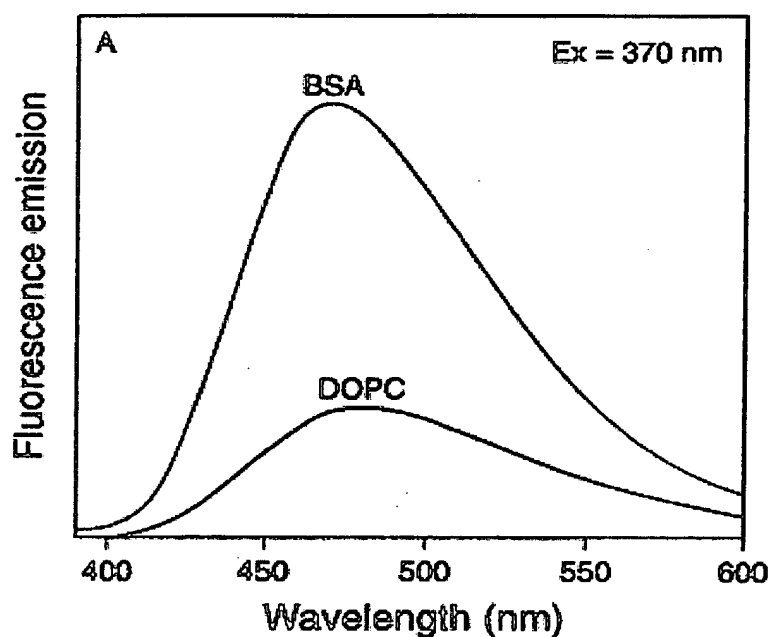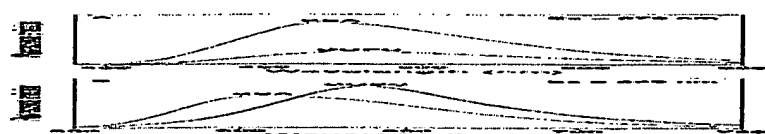
Figure 13.58 P248 6-propionyl-2-dimethylaminonaphthalene.

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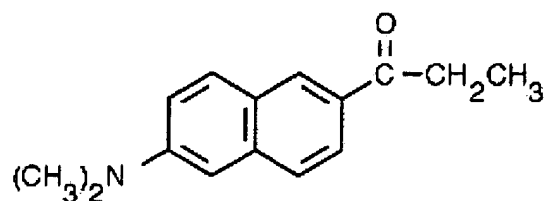
Figure 13.59 Normalized emission spectra of prodan (P248) excited at 345 nm in **1)** cyclohexane, **2)** dimethylformamide, **3)** ethanol and **4)** water.

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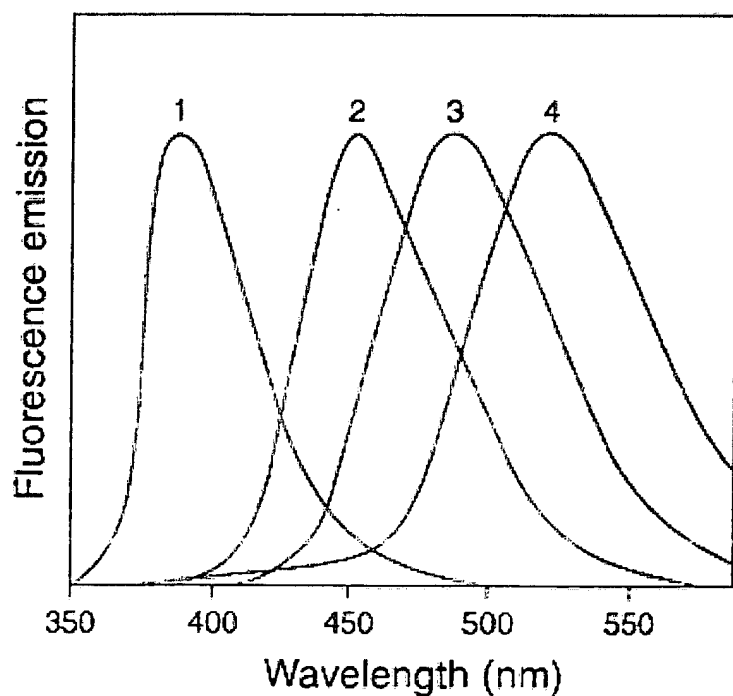
Figure 13.60 D250 6-dodecanoyl-2-dimethylaminonaphthalene.

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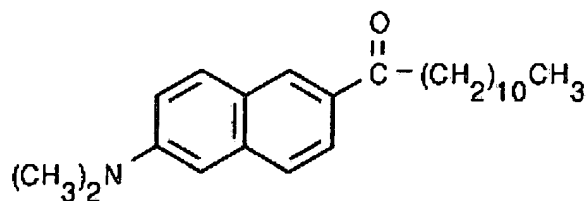
Figure 13.61 Normalized fluorescence emission spectra of Dapoxyl (2-aminoethyl)sulfonamide excited at 390 nm (D10460) in: 1) hexane, 2) chloroform, 3) acetone, 4) acetonitrile and 5) 1:1 acetonitrile/water.

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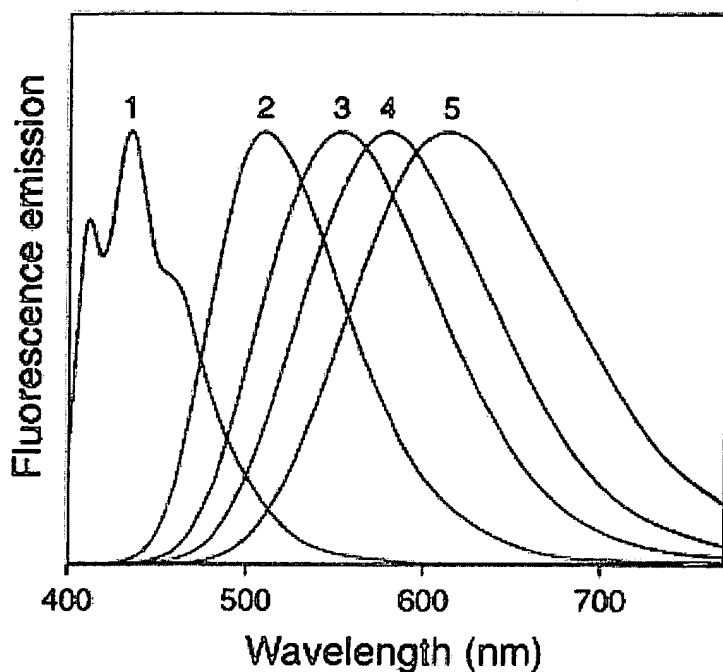
Figure 13.62 D12801 DISCONTINUED Dapoxyl butylsulfonamide.

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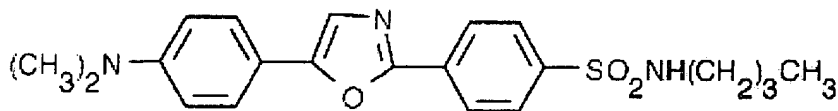
Figure 13.63 A47 1-anilinonaphthalene-8-sulfonic acid.


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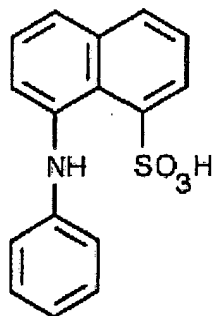
Figure 13.64 B153 bis-ANS.

$2K^+$

* * * * *
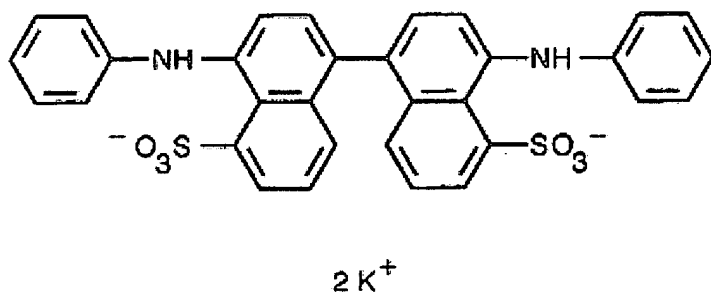
Product List — 13.5 Other Nonpolar and Amphiphilic Probes

Cat #	Product Name	Unit Size
A11760	4-amino-4'-benzamidostilbene-2,2'-disulfonic acid, disodium salt (MBDS)	100 mg
A47	1-anilinonaphthalene-8-sulfonic acid (1,8-ANS) *high purity*	100 mg
A50	2-anilinonaphthalene-6-sulfonic acid (2,6-ANS)	100 mg
B153	bis-ANS (4,4'-dianilino-1,1'-binaphthyl-5,5'-disulfonic acid, dipotassium salt)	10 mg

B3932	(E,E)-3,5-bis-(4-phenyl-1, 3-butadienyl)-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY® 665/676)	5 mg
B311	1,3-bis-(1-pyrenyl)propane	25 mg
D12800	Dapoxyl® sulfonic acid, sodium salt	10 mg
D3923	4-(dicyanovinyl)julolidine (DCVJ)	25 mg
D3922	4,4-difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-s-indacene (BODIPY® 493/503)	10 mg
D3921	4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (BODIPY® 505/515)	10 mg
D202	1,6-diphenyl-1,3,5-hexatriene (DPH)	100 mg
D109	5-dodecanoylaminofluorescein	100 mg
D250	6-dodecanoyl-2-dimethylaminonaphthalene (laurdan)	100 mg
F3857	fluorescein octadecyl ester	10 mg
H22730	4-heptadecyl-7-hydroxycoumarin	10 mg
H110	5-hexadecanoylaminofluorescein	100 mg
N1142	nile red	25 mg
O246	octadecyl rhodamine B chloride (R18)	10 mg
P459	3-(4-(6-phenyl)-1,3,5-hexatrienyl)phenylpropionic acid (DPH propionic acid)	25 mg
P35901	O-pivaloyloxymethyl umbelliferone (C-POM) *lipase substrate* *special packaging*	5 x 100 µg
P248	6-propionyl-2-dimethylaminonaphthalene (prodan)	100 mg
T53	2-(p-toluidinyl)naphthalene-6-sulfonic acid, sodium salt (2,6-TNS)	100 mg
T204	1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene p-toluenesulfonate (TMA-DPH)	25 mg

* * * * *

Data Table — 13.5 Other Nonpolar and Amphiphilic Probes

Cat #	MW	Storage	Soluble	Abs	EC	Em	Solvent	Notes	
A47	✲ ⋒	299.34	L	pH >6, DMF	372	7,800	480	MeOH	<1>
A50	✲	299.34	L	DMF	319	27,000	422	MeOH	<1>
A11760	✲	518.47	L	H_2O	342	37,000	450	pH 7	
B153	✲ ⋒	672.85	L	pH >6	395	23,000	500	MeOH	<1> <2>
B162	✲	542.76	L	MeCN, $CHCl_3$	345	69,000	378	$CHCl_3$	<3>
B311	✲	444.57	L	MeCN, $CHCl_3$	344	80,000	378	MeOH	<3>
B3932	✲	448.32	F,L	DMSO, $CHCl_3$	665	161,000	676	MeOH	<4>
D69	✲	628.98	L	MeCN, DMF	486	27,000	543	MeOH	<5>
D109	✲	529.63	L	DMSO, EtOH	495	85,000	518	MeOH	<6>
D202	✲	232.32	L	DMF, MeCN	350	88,000	452	MeOH	<7> <8>
D250	✲	353.55	L	DMF, MeCN	364	20,000	497	MeOH	<9>
D3921	✲	248.08	F,L	EtOH, DMSO	502	98,000	510	MeOH	<4>
D3922	✲	262.11	F,L	EtOH, DMSO	493	89,000	504	MeOH	<4>
D3923	✲	249.31	L	DMF, DMSO	456	61,000	493	MeOH	
D7760	✲	452.08	L	DMSO, EtOH	394	18,000	494	MeOH	<6>

D12760		436.58	L	DMSO, EtOH	366	15,000	454	MeOH	<6:
D12800		366.37	L	DMSO, H$_2$O	358	25,000	517	MeOH	<1(
F3857		584.79	L	DMSO, EtOH	504	95,000	525	MeOH	<6:
H110		585.74	L	DMSO, EtOH	497	92,000	519	MeOH	<6:
H1387		517.24	L	DMSO, EtOH	357	17,000	488	MeOH	<11
H22730		400.60	L	DMSO, EtOH	366	20,000	453	MeOH	<6:
N1142		318.37	L	DMF, DMSO	552	45,000	636	MeOH	<12
O246		731.50	F,DD,L	DMSO, EtOH	556	125,000	578	MeOH	<1:
O322		613.79	L	DMSO, EtOH	497	84,000	519	MeOH	<6:
O3852		658.89	L	DMSO, EtOH	497	80,000	519	MeOH	<6:
P65		219.29	L	MeOH, DMF	337	8,100	426	MeOH	
P80		304.30	L	DMF	344	42,000	376	MeOH	<1∠ <1!
P244		274.36	L	DMF, CHCl$_3$	341	41,000	376	MeOH	<1!
P248		227.31	L	DMF, MeCN	361	18,000	498	MeOH	<9:
P394		344.50	L	DMF, CHCl$_3$	341	42,000	377	MeOH	<1!
P459		304.39	L	DMF, DMSO	354	82,000	430	MeOH	<7:
P3900		503.70	D,L	DMF, DMSO	354	85,000	429	MeOH	<7:

| T53 | ✿ | 335.35 | L | DMF | 318 | 26,000 | 443 | MeOH | <1: |
| T204 | ✿ | 461.62 | D,L | DMF, DMSO | 355 | 75,000 | 430 | MeOH | <7: |

Notes

1. Fluorescence quantum yields of ANS and its derivatives are environment-dependent and are particularly sensitive to the presence of water. QY of A47 is about 0.4 in EtOH, 0.2 in MeOH and 0.004 in water. Em is also somewhat solvent-dependent (Biochim Biophys Acta 694, 1 (1982)).
2. B153 is soluble in water at 0.1–1.0 mM after heating.
3. Absorption spectra of bis-pyrenyl alkanes have additional peaks at ~325 nm and <300 nm. Emission spectra include both monomer (~380 nm and ~400 nm) and excimer (~470 nm) peaks.
4. The absorption and fluorescence spectra of BODIPY derivatives are relatively insensitive to the solvent.
5. Fluorescence of NBD and its derivatives in water is relatively weak. QY and τ increase and Em decreases in aprotic solvents and other nonpolar environments relative to water (Biochemistry 16, 5150 (1977); Photochem Photobiol 54, 361 (1991)).
6. Spectra of this product are pH-dependent. Data listed are for basic solutions prepared in methanol containing a trace of KOH.
7. Diphenylhexatriene (DPH) and its derivatives are essentially nonfluorescent in water. Absorption and emission spectra have multiple peaks. The wavelength, resolution and relative intensity of these peaks are environment dependent. Abs and Em values are for the most intense peak in the solvent specified.
8. Stock solutions of DPH (D202) are often prepared in in tetrahydrofuran (THF). Long-term storage of THF solutions is not recommended because of possible peroxide formation in that solvent.
9. The emission spectrum of P248 is solvent-dependent. Em = 401 nm in cyclohexane, 440 nm in CHCl$_3$, 462 nm in MeCN, 496 nm in EtOH and 531 nm in H$_2$O (Biochemistry 18, 3075 (1979)). Abs is only slightly solvent dependent. The emission spectra of D250 in these solvents are similar to those of P248.
10. Em = 520 nm when bound to phospholipid bilayer membranes. Fluorescence in H$_2$O is weak (Em ~600 nm).
11. Em of H1387 is significantly shifted in other solvents (Biochemistry 22, 5714 (1983)).
12. The absorption and fluorescence spectra and fluorescence quantum yield of N1142 are highly solvent-dependent (J Lipid Res 26, 781 (1985); Anal Biochem 167, 228 (1987)).
13. This product is intrinsically a sticky gum at room temperature.
14. P80 fluorescence lifetime is 62 nanoseconds in water, 57 nanoseconds in phospholipid vesicles and 140 nanoseconds in cetyltrimethylammonium

micelles.

15. Pyrene derivatives exhibit structured spectra. The absorption maximum is usually about 340 nm with a subsidiary peak at about 325 nm. There are also strong absorption peaks below 300 nm. The emission maximum is usually about 376 nm with a subsidiary peak at 396 nm. Excimer emission at about 470 nm may be observed at high concentrations.

* * * * *

products

Return to Product Groups

FluoroFectin™ pH sensitive reagents

pH sensitive fluorescent liposomal reagents for studying the mechanisms of transfection

Applications using confocal or normal fluorescence microscopy or FACS:

- Follow the interaction of the DNA-liposome complexes with cells
- Follow intracellular processing, entry and release from endosomes
- Quantify the interaction of nucleic acids (DNA or oligonucleotides) with cationic liposomes
- Ascertain liposome/DNA complex stability
- Characterize and quantify the effect of cell growth media, serum and additives on these complexes

References Library

FluoroFectin™ Technology overview
Fluorescent transfection
Transfected cells database
Literature References
FluoroFectin™ 20 Green pH Sensitve Protocol
FluoroFectin™ 20 Red pH Sensitve Protocol
FluoroFectin™ 20 UV pH Sensitve Protocol
FluoroFectin™ 40 Green pH Sensitve Protocol
FluoroFectin™ 40 Red pH Sensitve Protocol
FluoroFectin™ 40 UV pH Sensitve Protocol
FluoroFectin™ 60 Green pH Sensitve Protocol
FluoroFectin™ 60 Red pH Sensitve Protocol
FluoroFectin™ 60 UV pH Sensitve Protocol

The electrostatic and structural features of DNA-lipid assemblies critically affect the efficiency of the complexes as vehicles for cellular delivery of nucleic acids(1,2,3,4,5,6,7). FluoroFectin pH sensitive reagents provide the researcher with powerful tools for monitoring changes in these key parameters.

Cationic lipids such as DOTAP form positively charged liposomes which interact with the negatively charged DNA molecules to form a stable complex. With FluoroFectin™ pH sensitive reagents, this interaction can be followed by observing a change in fluorescence intensity of the pH sensitive fluorophore.

FluoroFectin™	Maximum	Maximum	pH Sensitive	Laser

pH Sensitive	Excitation Wavelength (nm)	Emission Wavelength (nm)		Range
Green	507	528	Fluorescence intensity increases with increased pH	visible
Red	565	585	Fluorescence intensity decreases with increased pH	visible
UV	370* 405*	445 445	The ratio 405/370 increases with increased pH	UV

370 nm is the pH independent isosbestic point and 405 nm is the maximum excitation wavelength of the basic form of the probe.
The ratio of intensities 405/370 allows to quantify the degree of fluorophore ionization.

The FluoroFectin™ range of transfection reagents offers a choice of 3 different pH sensitive fluorophores:

FluoroFectin™ Green pH sensitive: Fluorescein-labeled liposomes
FluoroFectin Red pH sensitive: Liposomes labeled with a patented red fluorophore
FluoroFectin™ UV pH sensitive: Liposomes labeled with a patented blue fluorophore

For each fluorophore 3 different liposome formulations are available:

FluoroFectin™ 20: based on DOTAP
FluoroFectin™ 40: based on DOTAP/DOPE
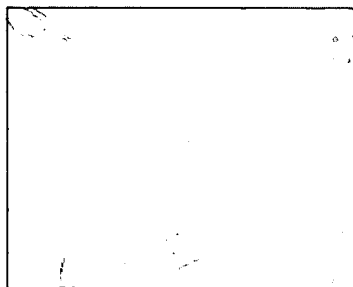FluoroFectin™ 60: based on DOTAP/Cholesterol

Figure 1
FluoroFectin™ pH Sensitive, NIH 3T3 fibroblast cells transfected with unlabeled oligonucleotide. Viewed with laser-scanning microscope (Zeiss Axiovert 135 M). Objective is Plan Neofluor 1.3 magnification 40, oil immersion.

Cat #	Description	Qty *
GDFG0100	FluoroFectin™ 20 Green pH sensitive	500
GDFS0100	FluoroFectin™ 20 Red pH Sensitive	500

GDFU0100	FluoroFectin™ 20 UV pH Sensitive	500
GDFG0200	FluoroFectin™ 40 Green pH sensitive	500
GDFS0200	FluoroFectin™ 40 Red pH Sensitive	500
GDFU0200	FluoroFectin™ 40 UV pH sensitive	500
GDFG0400	FluoroFectin™ 60 Green pH sensitive	500
GDFS0400	FluoroFectin™ 60 Red pH Sensitive	500
GDFU0400	FluoroFectin™ 60 UV pH Sensitive	500

Number of transfections in 24-well plates

Storage: +4°C

Other transfection applications possible with FluoroFectin™

☐ Fluorescent transfection
☐ *in vivo* transfection
☐ Oligonucleotides delivery

FluoroFectin™ Product pages

☐ FluoroFectin™Starter Kits

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Acknowledgements and Licensing Information
The FluoroFectin™ range of fluorescent transfection reagents has been developed for Qbiogene, for research use only, in the laboratory of Professor Y. Barenholz, Laboratory of Membrane and Lipid Research, Hebrew University-Hadassah Medical School, Jerusalem, Israel. Patents are pending on the U/V and pH-sensitive fluorophore and on applications of the Green.